



PATENT  
3587-0111P

IN THE U.S. PATENT AND TRADEMARK OFFICE

Applicant: MAY, Choo Yuen et al. Conf.: Unassigned  
Appl. No.: 10/642,597 Group: Unassigned  
Filed: August 19, 2003 Examiner: UNASSIGNED  
For: RECOVERY OF PALM PHYTONUTRIENTS

L E T T E R

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

October 22, 2003

Sir:

Under the provisions of 35 U.S.C. § 119 and 37 C.F.R. § 1.55(a), the applicants hereby claim the right of priority based on the following application:

<u>Country</u>	<u>Application No.</u>	<u>Filed</u>
MALAYSIA	PI 20023068	August 20, 2002

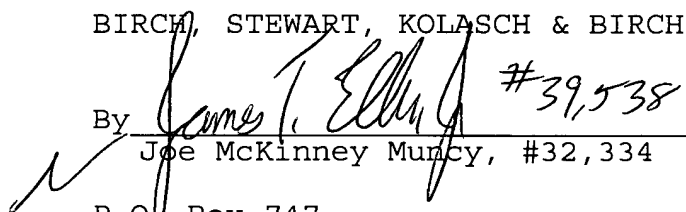
A certified copy of the above-noted application is attached hereto.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fee required under 37 C.F.R. §§ 1.16 or 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By

 #39,538  
Joe McKinney Muncy, #32,334

P.O. Box 747  
Falls Church, VA 22040-0747  
(703) 205-8000

KM/mzk  
3587-0111P

Attachment



**Perbadanan Harta Intelek Malaysia**  
**Intellectual Property Corporation of Malaysia**

Aras 27, 30 dan 32, Menara Dayabumi, Jalan Sultan Hishamuddin, 50623 Kuala Lumpur, Malaysia

Tel : 603-2274 2100 Fax : 603-2274 1332 Website : www.mipc.gov.my

*3537-01110*  
*10/11/02, 597*  
*AUGUST 19, 2003*  
*MA, CHOO Kuen et al.*  
*SSR2 (2002) 505-8000*

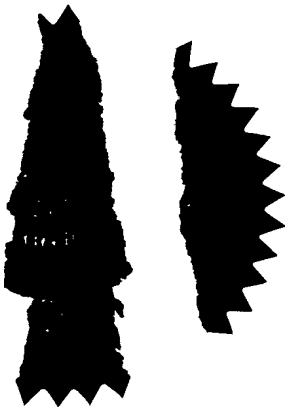
To:

**P. KANDIAH**

KANDIAH & ASSOCIATES SDN. BHD.  
Suite 8 - 7 -2, Menara Mutiara Bangsar  
Jalan Liku, Off Jalan Bangsar,  
59100 Kuala Lumpur  
**MALAYSIA**

**PATENT APPLICATION NO: PI 2002 3068**

This is to certify that annexed hereto is a true copy from the records of the Registry of Trade Marks and Patents, Malaysia of the application as originally filed which is identified therein.



By authority of the  
REGISTRAR OF PATENTS

  
**ABDUL RAHMAN RAMLI**  
(CERTIFYING OFFICER)  
4 September 2003



**KEMENTERIAN PERDAGANGAN DALAM NEGERI  
DAN HAL EHWAL PENGGUNA MALAYSIA**  
BAHAGIAN HARTA INTELEK,  
TINGKAT 27 & 32,  
MENARA DAYABUMI,  
JALAN SULTAN HISHAMUDDIN,  
50654 KUALA LUMPUR.  
*Ministry of Domestic Trade and Consumer Affairs Malaysia  
Intellectual Property Division.*

*Telefon : 03 - 22742100  
Fax : 03 - 22741332*


---

**CERTIFICATE OF FILING**

**APPLICANT** : MALAYSIAN PALM OIL BOARD (MPOB)  
**APPLICATION NO** : PI 20023068  
**REQUEST RECEIVED ON** : 20/08/2002  
**FILING DATE** : 20/08/2002  
**AGENT'S/APPLICANT'S FILE REF.** : PK/P867/MPOB/2002

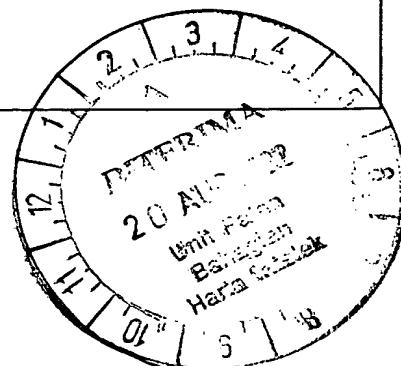
Please find attached, a copy of the Request Form relating to the above application, with the filing date and application number marked thereon in accordance with Regulation 25(1).

**Date** : 29/08/2002

  
-----  
(ROZILEE B. ASID)  
**for Registrar of Patents**

**To** : P. KANDIAH  
C/O KANDIAH & ASSOCIATES SDN BHD,  
SUITE 8-7-2, MENARA MUTIARA BANGSAR,  
JALAN LIKU, OFF JALAN BANGSAR,  
59100-KUALA LUMPUR  
MALAYSIA

<b>Patents Form N . 1</b> <b>PATENTS ACT 1983</b>  <b>REQUEST FOR GRANT OF PATENT</b> <b>(Regulation 7(1))</b>  <b>To: The Registrar of Patents</b> <b>Patent Registration Office</b> <b>Kuala Lumpur</b> <b>Malaysia</b>	<b>For Official Use</b> <i>Application No: PK/P867/MPOB/2002</i> <b>APPLICATION RECEIVED ON:</b> <i>20-08-2002</i>  <b>Fee received on:</b> <i>20-08-2002</i> <b>Amount:</b> <i>120000</i>  <b>*Cheque / Postal Order / Money Order / Draft /</b> <b>Cash No.:</b> <i>120000</i>		
<b>Please submit this Form in duplicate together with the prescribed fee.</b>	<b>Applicant's file reference: PK/P867/MPOB/2002</b>		
<b>THE APPLICANT(S) REQUEST(S) THE GRANT OF A PATENT IN RESPECT OF THE FOLLOWING PARTICULARS:</b> <b>TITLE OF INVENTION: RECOVERY OF PALM PHYTONUTRIENTS</b>			
<b>II. APPLICANT(S) (the data concerning each applicant must appear in this box or, if the space is sufficient, in the space below)</b>  <b>Name: MALAYSIAN PALM OIL BOARD (MPOB)</b>  <b>H.S.C./Passport No:</b>  <b>Address: 6, Persiaran Institusi, Bandar Baru Bangi</b> <b>43000 Kajang, Selangor, Malaysia</b>  <b>Address for service in Malaysia: Suite 8-7-2</b> <b>Menara Mutiara Bangsar</b> <b>Jalan Riong, Bangsar</b> <b>59100 Kuala Lumpur</b> <b>Tel: 2284 7872 Fax: 2284 1125</b>  <b>Nationality: A Company Established Under the Laws of Malaysia.</b>  <ul style="list-style-type: none"> <li>Permanent residence or principal place of business:  <b>AS ABOVE</b> </li> </ul> <table border="0" style="width: 100%;"> <tr> <td style="text-align: center;"> <b>Telephone Number</b>          (if any)   <b>2284 7872</b> </td> <td style="text-align: center;"> <b>Fax Number</b>          (if any)   <b>2284 1125</b> </td> </tr> </table>		<b>Telephone Number</b> (if any)  <b>2284 7872</b>	<b>Fax Number</b> (if any)  <b>2284 1125</b>
<b>Telephone Number</b> (if any)  <b>2284 7872</b>	<b>Fax Number</b> (if any)  <b>2284 1125</b>		
<b>Additional Information (if any)</b>			



20023068

III. INVENTOR

Applicant is the inventor:

Yes ☐

No ☒

If the applicant is not the inventor:

Name of the inventors: 1. Dr. Choo Yuen May (IC No. 551227-07-5578)  
2. Harrison Lau Lik Nang (IC No. 750810-13-5883)  
3. PUAH CHIEW WEI ( 761019-14-6322)  
4. Dr. Ma Ah Ngan (IC No. 480517-08-5357)  
5. Dr. Yusof Barison (IC No. 480620-05-5359)

Address of inventors:

All are of  
6, Persiaran Institusi, Bandar Baru Bangi  
43000 Kajang, Selangor, Malaysia

A statement justifying the applicant's right to the patent accompanies this Form:

Yes ☒

No ☐

Additional Information (if any)

IV. AGENT OR REPRESENTATIVE:

Applicant has appointed a patent agent in accompanying Form No. 17. (to follow)

Yes ☐

No ☐

Agent's Registration No:

PA 90/019

Applicants have appointed P. Kandiah to be their common representative

IV. DIVISIONAL APPLICATION

This application is a divisional application

Yes ☐

No ☐

The benefit of the

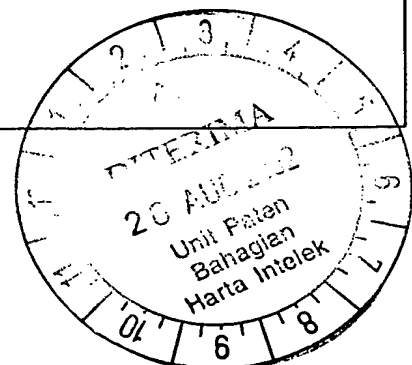
Filing date

Priority date

Of the initial application is claimed in as much as the subject matter of the present application is contained in the initial application identified below: -

Initial Application No:

Date of filing of initial application:



71-123068

VI. DISCLOSURES TO BE DISREGARDED FOR PRIOR ART PURPOSES

Additional information is contained in supplemental box:

- (a) Disclosure was due to acts of applicant or his predecessor in title

Date of disclosure:

- (b) Disclosure was due to abuse of rights of applicant or his predecessor in title

Date of disclosure:

A statement specifying in more detail the facts concerning the disclosure accompanies this Form

Yes ☐

No ☐

Additional Information (if any)

VII. PRIORITY CLAIM (if any)

The priority of an earlier application is claimed as follows :

Country (if the earlier application is a regional or international application, indicate the office with which it is filed):

Filing Date:

Application No:

Symbol of the International Patent Classification:

If not yet allocated, please tick

☐

The priority of more than one earlier application is claimed:

Yes ☐

No ☐

The certified copy of the earlier application(s) accompanies this Form:

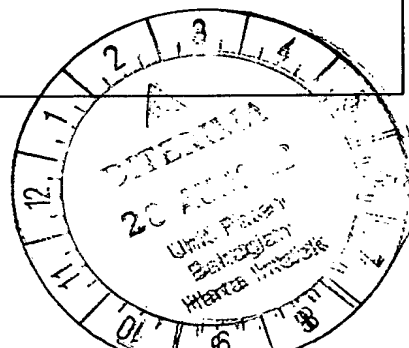
Yes ☐

No ☐

If No, it will be furnished by (if requested by Registrar)

Additional Information (if any) :

00023038



VIII. CHECK LIST

A. This application contains the following:

1.	Request	1	Sheets
2.	Description	21	Sheets
3.	Claim	3	Sheets
4.	Abstract	1	Sheets
5.	Drawing	-	Sheets
	Total	26	Sheets

B. This Form, as filed, is accompanied by the items checked below:

(a)	signed Form No. 17	<input type="checkbox"/>
(b)	declaration that inventor does not wish to be named in the patent	<input type="checkbox"/>
(c)	statement justifying applicant's right to the patent	<input type="checkbox"/>
(d)	statement that certain disclosures be disregarded (to follow)	<input type="checkbox"/>
(e)	priority document (certified copy of earlier application)	<input type="checkbox"/>
(f)	cash, cheque, money order, banker's draft or postal order for the payment of application fee	<input checked="" type="checkbox"/>
(g)	other documents (specify) -	<input type="checkbox"/>

IX. SIGNATURE:

*P. Kandiah*  
P. Kandiah  
\*\*(Applicant/Agent)

\_\_\_\_\_  
(Date)

If Agent, indicates Agent's Registration No: PA 90/019

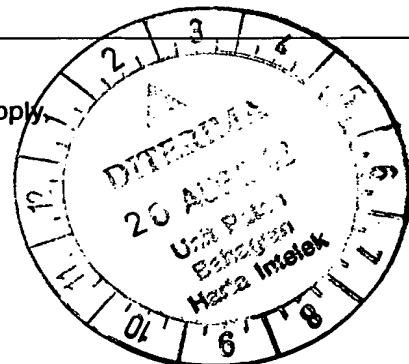
For Official Use

1. Date application received:
2. Date of receipt of correction, later filed papers or drawings completing the application:

\* Delete whichever does not apply.

\*\* Type name under signature and delete whichever does not apply.

20023068



## RECOVERY OF PALM PHYTONUTRIENTS

### **FIELD OF INVENTION**

- 5 This invention relates to a process of recovery of phytonutrients such as carotenes, phospholipids and ubiquinones using vacuum distillation, various physical and chemical treatments and purification of the phytonutrients containing natural esterified oils and fats and has particular but not exclusive application to their recovery from palm oil.

### 10 **BACKGROUND ART**

- Carotenoids are the natural pigments, which impart a rich orange-red colour in plants and animals. Carotenoids are found in abundance (~600 types) in nature. These include beta-carotene and alpha-carotene, which can be converted into Vitamin A (retinal) in the body. Other non-vitamin A carotenoids includes lycopene and phytoene. All these are present in crude palm oil. In fact, crude palm oil is one of the richest natural plant sources with carotenes with concentration of 500-700 ppm. Carotenoids have a number of important physiological properties. For example lycopene suppresses the growth of various cancer lines. These include the lung and liver cancer as well as colon tumours.

- Ubiquinone (Coenzyme Q10) is a naturally occurring coenzyme found in palm oil. The concentration of ubiquinone in crude palm oil is determined in the range of 10-100ppm (Hazura et al. 1990). Ubiquinone is found mostly in the inner mitochondrial membrane, especially in the heart, liver, kidney and pancreas. It plays an important role in the mitochondrial electron transport chain and is also a powerful antioxidant and free radical's scavenger, and it is believed to possess membrane-stabilising properties. Since its discovery, ubiquinone has been used to aid in the treatment of many cardiovascular diseases such as congestive heart failure, cardiac arrhythmias and hypertension.

Phospholipids are essential for cell membrane repair, optimum mental function (it provides vital neurotransmitter precursor) and lipid metabolism. Phospholipids (phosphatides) are indispensable components of cell membranes and are also natural emulsifiers, helping fats dissolve in water. They support a healthy cardiovascular system and have been used as a fat emulsifier in preventing arteriosclerosis, cardiovascular disease, brain function, and proper nerve function and maintain proper electrical energy and nutrients transfer across the cell membrane.

A number of patents have been filed on the recovery of carotenes from palm oil. These include US5157132, GB2160874, US6072092 and EP0349138. The recovery processes employ esterification/ transesterification, molecular distillation, adsorbent at some stages. The current process is an advanced process integrating steps of at least one stage vacuum distillation; various physical and chemical treatment and purification to the phytonutrients concentrates. The integrated process yields higher carotenes concentration enriched with ubiquinones in indigenous diacylglycerols; and phospholipids enriched fraction.

### **SUMMARY OF INVENTION**

This present invention relates to a process for the recovery of carotene concentrates such as carotenes, ubiquinones, and phospholipids from natural esterified oils and fats has in particular but not exclusive to crude palm oil and palm oil products.

This process involves the integration steps of (i) at least one stage vacuum distillation at temperatures ranging 80<sup>0</sup>C-220<sup>0</sup>C and pressure less than 40mTorr; (ii) various physical and chemical treatment including filtration, solvent partitioning, saponification re-transesterification; and (iii) purification of phytonutrients containing concentrate.

Esterification / transesterification of crude palm oil and degummed and bleached palm oil is carried out with alkyl alcohol in the presence of an alkaline catalyst under conditions sufficient to convert free fatty acids and acylglycerols into alkyl esters-rich layer is either subjected to another re-transesterification process or clean water wash for neutralisation. The esterified palm oil is subjected to one or multi-stage vacuum distillation.

After first vacuum distillation, the carotenes enriched alkyl esters (residue) is subjected to the re-transesterification process. The process is carried out with alkyl alcohol with catalyst dissolving in alcohol or clean water under sufficient conditions to convert the traces of acylglycerols into alkyl esters and glycerol. The re-transesterified alkyl esters-rich layer is then subjected to second vacuum distillation for the production of carotenes concentrate.

In some instances, the esterified and or re-esterified palm oil is subjected to one stage vacuum distillation, yielding a concentrate residue enriched in carotenes.

The carotenes enriched alkyl esters layer from the first vacuum distillation is filtered or treated with hydrocarbon solvent to remove monoacylglycerols. The filtrate is subjected to second vacuum distillation for the production of carotenes concentrate.

Mixture of carotenes concentrate could also be produced by second stage vacuum distillation alone under conditions without going through third stage vacuum distillation.

A minimum amount of palm oil ethyl esters is added to the treated carotenes enriched alkyl esters (methyl esters in this case) prior to further vacuum distillation. Carotenes concentrate produced is enriched with ubiquinones in diacylglycerols with phospholipids. Treatment of carotenes concentrate is carried out using hydrophobic and hydrophilic solvents for further purification. The concentrate could be saponified to

obtain desired concentration of carotenes fractions. Phospholipids are also recovered by membrane filtration of crude palm oil prior to conversion of oil into alkyl esters.

This present invention has many advantages. It is an integrated process where  
5 carotenes are recovered from crude palm oil, and, degummed and bleached palm oil. Carotenes recovered from this process present in diacylglycerols which is an effective carrier and dietary oil. With the improved two stage vacuum distillation, various treatments can be incorporated between the distillation stages. For instance, indigenous monoacylglycerols can be removed from the residue of first vacuum distillation after ten  
10 times of concentration and recovered as a high purity co-product. Other valuable minor components, ubiquinone and phospholipids are being concentrated in carotenes concentrate during the process.

15

20

25

## **DETAILED DESCRIPTION OF THE INVENTION**

### **Example 1**

Crude palm oil (CPO) was esterified by using sodium hydroxide as catalyst with  
5 methanol to produce CPO methyl ester (ME). Glycerol was drained and CPOME was  
washed with hot distilled water. The neutralised CPOME was subjected to molecular  
distillation at temperature of 110°C, wiper speed of 250rpm and pressure of 5mTorr.  
Residue and distillate were collected for analysis of carotenes content. The carotenes  
concentration was 6.5% with recovery of 80.5%. Detail results are shown in the Table 1.

10

### **Example 2**

Bleached and degummed palm oil (BDPO) was esterified by using sodium hydroxide  
with methanol to produce BDPO methyl ester (ME). Glycerol was drained and BDPOME  
was washed with hot distilled water. The neutralized BDPOME was subjected to  
15 molecular distillation at temperature of 130°C, wiper speed of 250rpm and pressure of  
5mTorr. Residue and distillate were collected for analysis of carotenes content. The  
carotenes concentration was 12.9% with recovery of 92.5% was obtained. Detailed  
results are shown in the Table 2.

20

### **Example 3**

Crude palm oil (CPO) was esterified by using sodium hydroxide with methanol to  
produce CPO methyl ester (ME). Glycerol was drained and CPOME was washed with  
hot distilled water. The neutralised CPOME was subjected to molecular distillation at  
temperature of 150°C, wiper speed of 300rpm and pressure of 30mTorr. All samples  
25 were analysed for carotenes content. The carotenes concentration was 5.9% with  
recovery of 79.9%. Detail results are shown in the Table 3.

Example 4

Bleached and degummed palm oil (BDPO) was esterified by using sodium hydroxide with methanol to produce BDPO methyl ester (ME). Glycerol was drained and BDPOME was washed with hot distilled water. The neutralized BDPOME was subjected to  
 5 molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 5mTorr. Residue was collected and analysed for carotenes content. The carotenes concentration was 8.5% with recovery of 91.7%. Detailed results are shown in the Table 4.

10 Example 5

Bleached and degummed palm oil (BDPO) was esterified by catalytic reaction with methanol to produce BDPO methyl ester (ME). Glycerol was drained and BDOME was washed with hot distilled water. The neutralised BDOME was subjected to 1<sup>st</sup> molecular distillation at temperature of 110°C, wiper speed of 250rpm and pressure of 3mTorr.  
 15 Residue was subjected to 2<sup>nd</sup> molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 3mTorr. All samples were analysed for carotenes content. The carotenes concentration was 8.6% with recovery of 86%. Detail results are shown in the Table 5.

20 Example 6

Crude palm oil (CPO) was esterified by catalytic reaction with methanol to produce CPO methyl ester (ME). Glycerol was drained and CPOME was washed with hot distilled water. The neutralised CPOME was subjected to fast speed molecular distillation at temperature of 90°C, wiper speed of 250rpm and pressure of 20mTorr. Residue was re-  
 25 transesterified to obtain higher degree of methyl esters conversion. The re-transesterification was carried out using sodium methyllate as the catalyst. Treated sample was subjected to 2<sup>nd</sup> molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 3mTorr. The samples were analysed for carotenes and ubiquinone

content. The carotenes concentration was 14.4% with recovery of 92.7% and ubiquinone concentration was 0.3% with recovery of 94.7%. Detail results are shown in the Table 6.

#### Example 7

- 5 Bleached and degummed palm oil (BDPO) was esterified by catalytic reaction with methanol to produce BDPO methyl ester (ME). Glycerol was drained and BDPOME was washed with hot distilled water. The neutralised BDPOME was subjected to fast speed molecular distillation at temperature of 90°C, wiper speed of 200rpm and pressure of 20mTorr. Residue was treated with hexane (1:1, v/v) and chilled to 0°C for two hours.
- 10 The mixture was filtered and pumped dried. Treated residue was subjected to 2<sup>nd</sup> molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 5mTorr. All samples were analysed for carotenes content. The carotenes concentration was 12.2% with recovery of 87.9%. Detailed results are shown in the Table 7.

#### 15 Example 8

- Crude palm oil (CPO) was esterified by catalytic reaction with methanol to produce CPO methyl ester (ME). Glycerol was drained and CPOME was washed with hot distilled water. The neutralised CPOME was subjected to fast speed molecular distillation at temperature of 90°C, wiper speed of 200rpm and pressure of 20mTorr. Residue was
- 20 treated with hexane (1:1, v/v) and chilled to 0°C for two hours. The mixture was filtered and washed with MeOH/H<sub>2</sub>O (5:2.5:0.5, v/v/v) for two times followed by vacuum pumped dried. Treated sample was subjected to 2<sup>nd</sup> molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 5mTorr. All samples were analysed for carotenes content. The carotenes concentration was 18.1% with recovery of 87.9%. Detailed results
- 25 are shown in the Table 8.

### Example 9

Crude palm oil (CPO) was esterified by catalytic reaction with methanol to produce CPO methyl ester (ME). Glycerol was drained and CPOME was washed with hot distilled water. The neutralised CPOME was subjected to fast speed molecular distillation at temperature of 90°C, wiper speed of 200rpm and pressure of 20mTorr. Residue was treated with iso-octane (1:1, v/v) and chilled to 0°C for two hours. The mixture was filtered and pumped dry. Treated sample was subjected to 2<sup>nd</sup> molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 5mTorr. All samples were analysed for carotenes content. The carotenes concentration was 11.0% with recovery of 88.3%. Detail results are shown in the Table 9.

### Example 10

Crude palm oil (CPO) was esterified by catalytic reaction with methanol to produce CPO methyl ester (ME). Glycerol was drained and CPOME was washed with hot distilled water. The neutralised CPOME was subjected to fast speed molecular distillation at temperature of 90°C, wiper speed of 200rpm and pressure of 20mTorr. The residue was then subjected to re-esterification process, 50g of the concentrate was re-transesterified with 1 % alkaline catalyst (NaOH) dissolved in 20ml methanol. The mixture was refluxed at 60 - 65°C for 100 minutes. The sample of the re-esterification process was analysed for total carotenes, esters, acylglycerols and other minor components. The results of the analysis were shown in Table 10.

### Example 11

The CPOME produced subjected to similar process to that of Example 10. The product produced was then subjected to re-esterification process, 50g of the concentrate was re-transesterified with 1% sodium hydroxide dissolved in 5ml distilled water. The mixture was refluxed at 60 - 65°C for 30 minutes. The sample of the re-esterification process was analysed for total carotenes, esters, acylglycerols and other minor components. The results of the analysis were shown in Table 11.

### Example 12

Residue from fast speed molecular distillation of CPOME (Example 8) was added with 10% (v/v) CPO ethyl esters. The mixture was subjected to 2<sup>nd</sup> molecular distillation at temperature of 150°C, wiper speed of 250rpm and pressure of 1mTorr. The mass flow rate of the mixture in the distillation processes has increased 3 times of the normal flow rate without addition of ethyl esters. All samples were analysed for carotenes content. The carotenes concentration was 12.8% with recovery of 87.4%. Detailed results are shown in Table 12.

### 10 Example 13

5.0g of carotenes concentrate was subjected to unsaponification with 7.0ml of 10% potassium hydroxide in 30.0ml of ethanol. The mixture was refluxed for ½ hour. The reacted mixture was transferred to a separating funnel and the unsaponifiable matters were extracted with 50 ml of hexane: distilled water (90:10, v/v) for 3 times. The extracts were neutralised with copious of 10% ethanol in distilled water. The neutralised extract was then vacuum pumped dry and analysed. The results of the analysis are shown in Table 13.

### Example 14

20 Carotenes concentrate (from Example 8) was used as crude material in the treatment. 0.1g of carotenes concentrate was added to 1 ml of Hexane and 3 ml of Methanol. The mixture was chilled to -10°C for 1 hour. The top and bottom layers were separated and vacuum pumped dried. Samples were analysed for total carotenes content. The carotenes concentration was 30.1% with recovery of 69%. Detail results are shown in the Table 14.

**Example 15**

Carotenes concentrate (from Example 8) was used as crude material in the treatment, 0.16g of carotenes concentrate was added to 5 ml of Hexane and 10ml of Methanol. The mixture was chilled to -10°C for 1 hour. The top and bottom layers were separated and vacuum pumped dry. Samples were analysed for total carotenes content. The carotenes concentration was 24.3% with recovery of 84.7%. Detail results are shown in the Table 15.

**Example 16**

Carotenes concentrate produced from examples 1, 3 and 4 were analysed for total phospholipids content. The results are shown in Table 16 with the concentration ranging from 0.60% to about 4.0%.

**Example 17**

2 litres of CPO was filtered with a membrane filter with a 0.05µm pore size. This process was carried out to reduce impurities in the CPO. These include phospholipids, iron and copper. The CPO was subjected to the membrane system with the temperature of 60°C, pressure of 2bar with 300rpm. The filtrate was analysed for total phospholipids. It was found that the total phospholipids could be reduced to 46.40ppm from 171.17ppm found in CPO.

**Example 18**

500g of neutralised palm oil (NPO) was esterified by sodium methylate with methanol to produce NPO methyl esters (ME). Glycerol was drained and the NPOME was divided into two portions for different neutralisation approaches. To the first part of NPOME, 10% of distilled water was used for each washing step until neutralised NPOME was obtained. To the second part of NPOME, hydrochloric acid was added into distilled water until pH 4-5. 10% of the acidified distilled water was then used for each washing step until NPOME was neutralised. The result shows that the acidified distilled water is

better than normal distilled water for neutralization of NPOME produced by reducing the total amount of distilled water used by 40%. All minor components such as carotenes, vitamin E, phytosterols and squalene were preserved well in acidified water washing. The results are shown in Table 18.

5

10

15

20

25

**Table 1 (Single Stage Distillation – No treatment)**

Condition: 110°C, 250rpm, 0.93ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters	571.0	246.7	100.0
Carotenes Concentrate	65232.6	198.6	80.5

**Table 2 (Single Stage Distillation – No treatment)**

Condition: 130°C, 250rpm, 0.93ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: BDPO Methyl Esters	571.0	246.7	100.0
Carotenes Concentrate	129159.0	228.2	92.5

**Table 3 (Single Stage Distillation – No treatment)**1<sup>st</sup> Distillation: 150°C, 300rpm, 0.93ml/min, 30mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters	698.0	603.1	100.0
Carotenes Concentrate	58695.0	481.8	79.9

**Table 4 (Single Stage Distillation – No treatment)**1<sup>st</sup> Distillation: 150°C, 250rpm, 0.93ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: BDPO Methyl Esters	571.0	246.7	100.0
Carotenes Concentrate	84819.0	226.2	91.7

**Table 5 (Two Stage Distillation – No treatment)**1<sup>st</sup> Distillation: 110°C, 250rpm, 0.9ml/min, 3mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: BDPO Methyl Ester	612.0	3172.6	100.0
Carotenes enriched alkyl esters	47174.0	2959.1	93.3

2<sup>nd</sup> Distillation: 150°C, 250rpm, 0.93ml/min, 3mTorr

	Carotene		
	ppm	mg	%Recovery
Carotenes enriched alkyl esters	47174.0	2794.4	100.0
Carotenes Concentrate	86625.0	2402.5	86.0

**Table 6 (Two Stage Distillation – Re-transesterification of concentrate after 1<sup>st</sup> Distillation)**1<sup>st</sup> Distillation: 90°C, 200rpm, 2.2ml/min, 20mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters	682.0	4124.7	100.0
Carotenes enriched alkyl esters	6796.0	4022.1	97.5

2<sup>nd</sup> Distillation: 150°C, 250rpm, 0.93ml/min, 3mTorr

	Carotene			Ubiquinone		
	ppm	mg	%Recovery	ppm	mg	%Recovery
Feed: Treated carotenes enriched alkyl esters	6790.0	2933.3	100.0	140	60.5	100.0
Carotenes Concentrate	143123.0	2720.5	92.7	3014.0	57.3	94.7

**Table 7 (Two Stage Distillation – Treatment with Hexane)**1<sup>st</sup> Distillation: 90°C, 200rpm, 2.2ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: BDPO Methyl Esters	571.0	2466.7	100.0
Carotenes enriched alkyl esters	3949.4	2149.7	87.1

2<sup>nd</sup> Distillation: 150°C, 250rpm, 0.93ml/min, 7mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: Treated carotenes enriched alkyl esters	3949.4	1023.7	100.0
Carotenes Concentrate	121825.0	900.2	87.9

**Table 8 (Two Stage Distillation – Treatment with Hexane and MeOH/H<sub>2</sub>O Washing)**1<sup>st</sup> Distillation: 90°C, 200rpm, 2.2ml/min, 20mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters	571.0	2466.7	100.0
Carotenes enriched alkyl esters	4991.5	2630.7	98.3

2<sup>nd</sup> Distillation: 150°C, 250rpm, 0.93ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: Carotenes enriched alkyl esters	4991.5	1293.8	100.0
Carotenes Concentrate	181075.6	1134.7	87.7

**Table 2 (Two Stage Distillation – Treatment with Iso-Octane)**1<sup>st</sup> Distillation: 90°C, 200rpm, 2.2ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters	602.0	2340.6	100.0
Carotenes enriched alkyl esters	3720.0	2105.2	89.9

2<sup>nd</sup> Distillation: 150°C, 250rpm, 0.93ml/min, 5mTorr

	Carotene		
	ppm	mg	%Recovery
Feed: Treated carotenes enriched alkyl esters	3720.0	964.2	100.0
Carotenes Concentrate	110481.0	851.8	88.3

Note:

MG  
DG  
TG  
N.D.  
CPO  
BDPO  
Other Minor Components

: Monoacylglycerol  
: Diacylglycerol  
: Triacylglycerol  
: Non-detectable  
: Crude Palm Oil  
: Bleached and Degummed Palm Oil  
: Squalene, Sterols, Tocols (tocopherols and tocotrienol)

**Table 10 (Re-transesterification of carotenes enriched alkyl esters after first stage distillation-catalyst dissolved in methanol)**

	Percentage (%)				
	Esters	MG	DG	TG	Others Minor Components
CPO Methyl esters	99.413	0.296	0.043	N.D.	0.177
Carotenes enriched alkyl esters	96.730	0.876	0.509	N.D.	1.253
Treated carotenes enriched alkyl esters	98.032	0.274	N.D.	N.D.	1.085

**Table 11 (Re-transesterification of carotenes enriched alkyl esters after first stage distillation-catalyst dissolved in treated water)**

	Percentage (%)				
	Esters	MG	DG	TG	Others Minor Components
CPO Methyl esters	99.413	0.296	0.043	N.D.	0.177
Carotenes enriched alkyl esters	96.730	0.876	0.509	N.D.	1.253
Treated carotenes enriched alkyl esters	97.740	0.263	0.019	N.D.	1.357

**Table 12 (Two Stage Distillation-with addition of ethyl esters)**2<sup>nd</sup> Distillation: 150°C, 250rpm, 1 mTorr, 3ml/min

	Carotenes		
	ppm	mg	%Recovery
Feed: CPO Methyl Esters + 10% Ethyl Esters	4991.5	998.3	100
Carotenes Concentrate	128120	872.7	87.4

**Table 13 (Saponification of carotenes concentrate)**

	Percentage (%)					
	FFA	Esters	MG	DG	TG	Others
Carotenes Concentrate	1.45	1.11	34.61	18.26	33.19	2.10
Unsaponified Sample	24.42	0.00	0.00	13.92	34.95	11.58
						15.13

**Table 14 (Partition of carotenes)**

	Carotenes		
	ppm	mg	%Recovery
Carotenes Concentrate	170982	0.0172	100.0
Hexane Layer	301060	0.0119	69.0
Methanol Layer	58556	0.0036	30.2

**Table 15 (Partition of carotenes)**

	Carotenes		
	ppm	mg	%Recovery
Carotenes Concentrate	170982	0.0278	100.0
Hexane Layer	243538	0.0235	84.7
Methanol Layer	41274	0.0027	11.6

**Table 16**

	Phospholipids (%)
Carotenes Concentrate (from Example 1)	1.78
Carotenes Concentrate (from Example 3)	3.83
Carotenes Concentrate (from Example 4)	0.78

**Table 18**

	Concentration (ppm)	
	Carotenes	Vitamin E
Neutralised Palm Oil	512	950
Acidified distilled water washed NPOME	508	908
Normal distilled water washed NPOME	500	921

**CLAIMS**

- 1) A process to recover carotene concentrates comprising the steps of:
- 5           i)       subjecting alkyl esters produced from palm oil to at least one stage vacuum distillation at temperature ranging from 80-220<sup>0</sup>C and pressure at less than 40mTorr to yield phytonutrients concentrate in residue;
- ii)       separation of polar lipids and other impurities from the residue in step (i);
- 10          iii)       subjecting the treated residue from step (ii) to a second vacuum distillation wherein residue from the distillation contain carotenes concentrates consisting carotenes, ubiquinones and phospholipids;
- 2) A process to recover carotene concentrates claimed in claim 1 wherein the second
- 15       vacuum distillation is carried out at temperature ranging from 80<sup>0</sup>C to 200<sup>0</sup>C and at pressure less than 40mTorr.
- 3) A process to recover carotene concentrates as claimed in claim 1 wherein the separation of polar lipids and other impurities in step (ii) is done in any one of the
- 20       ways consisting of:
- i)       treating the residue in step (i) of claim 1 with a hydrocarbon solvent with or without subsequent alkyl alcohol/ treated water purification to remove the monoacylglycerols; or
- ii)       re-transesterifying the residue in step (i) of claim 1 using alkaline
- 25       catalysts to convert the traces of acylglycerols into alkyl esters and glycerol; or
- iii)       direct filtrating of residue in step (i) of claim 1 under vacuum.

- 4) A process to recover carotene concentrates as claimed in claim 3 wherein the mixture in step (i) is chilled down to low temperature for at least 2 hours and monoacylglycerols is separated from the residue.
- 5) A process to recover carotene concentrates as claimed in claim 3 wherein alkaline catalyst used in the re-transesterification in step (ii) is selected from a group consisting of such as sodium hydroxide, potassium hydroxide and sodium methylate in the presence of short and branched alkyl alcohol such as methanol and ethanol.
- 6) A process to recover carotene concentrates as claimed in claim 3 wherein 2% of palm oil ethyl esters are added to the treated residue in step (iii) prior to subsequent vacuum distillation.
- 7) A process to recover carotene concentrates as claimed in claim 1 wherein the carotenes concentrate is further purified by either:
- i) adding alkaline catalyst in presence of alkyl alcohol such as potassium hydroxide in ethanol; or
  - ii) adding hydrocarbon solvent and alkyl alcohol and chilled to  $-10^{\circ}\text{C}$  for at least one hour to partition the carotenes into hydrocarbon layer.
- 8) A process to recover carotene concentrates as claimed in claim 3 and 7 wherein the hydrocarbon solvents used are hexane or iso-octane and the alkyl alcohols used are short and branched alkyl alcohols such as methanol and ethanol.
- 9) A process to recover carotene concentrates as claimed in claim 1 wherein the alkyl esters is produced from the crude palm oil or treated palm oil such as bleached and degummed palm oil and membrane filtered palm oil.

- 10) A process to recover carotene concentrates as claimed in claim 9 wherein the removal of excess of alkaline catalyst in alkyl esters produced is carried out by using acidified water pH between 4-5.
- 5 11) Carotenes, ubiquinones, and phospholipids as produced in any of the claims 1 to 10.

10

15

20

25

## RECOVERY OF PALM PHYTONUTRIENTS

### ABSTRACT

- 5 A process for the recovery of phytonutrients such as carotenes, phospholipids and ubiquinones from palm oil esters is disclosed. This process comprises the steps of vacuum distillation, treatment and purification of concentrate containing these phytonutrients. The alkyl esters is subjected to at least one stage vacuum distillation at temperature from 80°C to 220°C and pressure less than 40mTorr. The carotenes  
10 concentrate is subjected to various physical and chemical treatments to yield higher carotenes concentration enriched with ubiquinones in indigenous diacylglycerols.